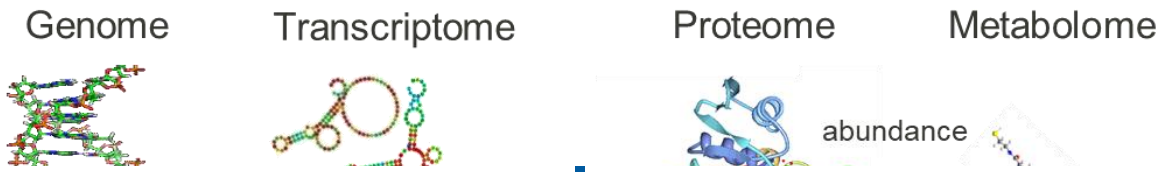
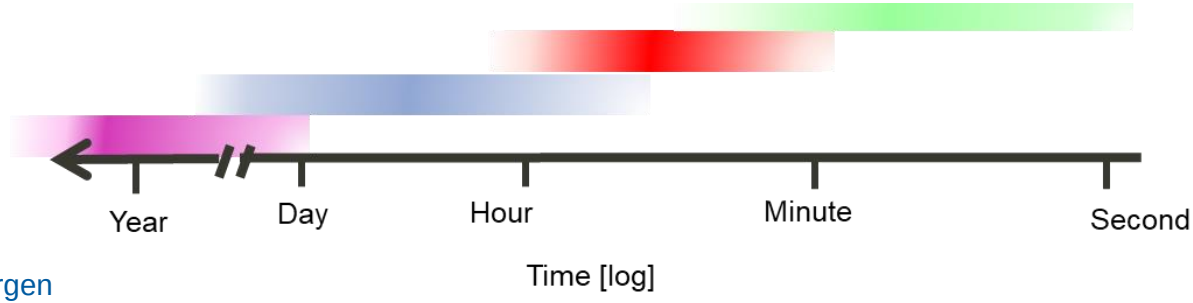
UFZ HELMHOLTZ Zentrum für Umweltforschung



Integration of multi-(meta)omics in cellular and microbiome toxicity



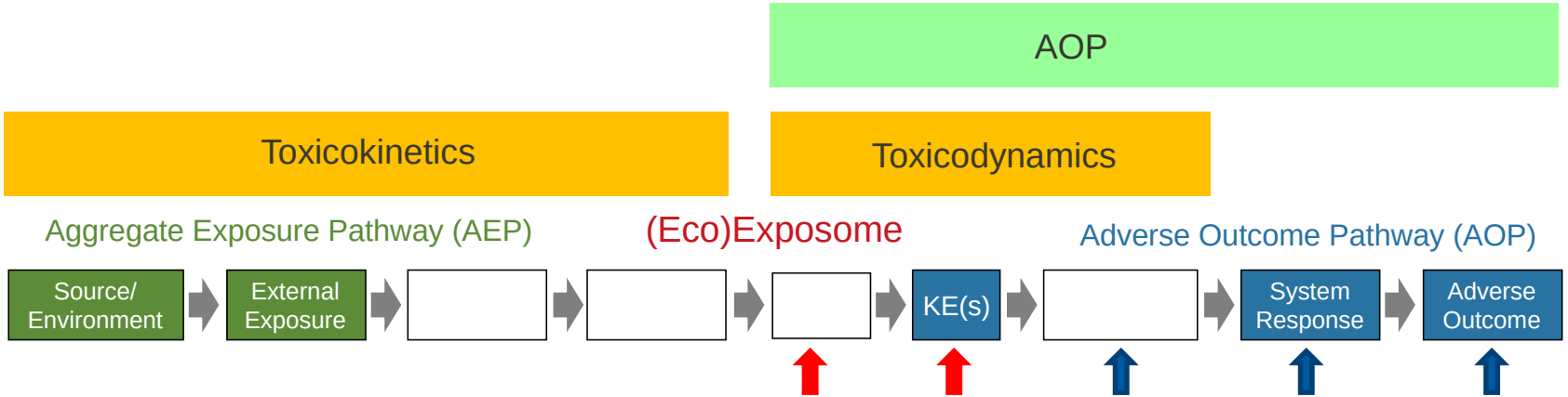
Prof. Dr. Martin von Bergen
Department Molecular Systems Biology

What is omics good for in toxicology?

https://share.america.gov/english-idiom-canary-coal-mine/

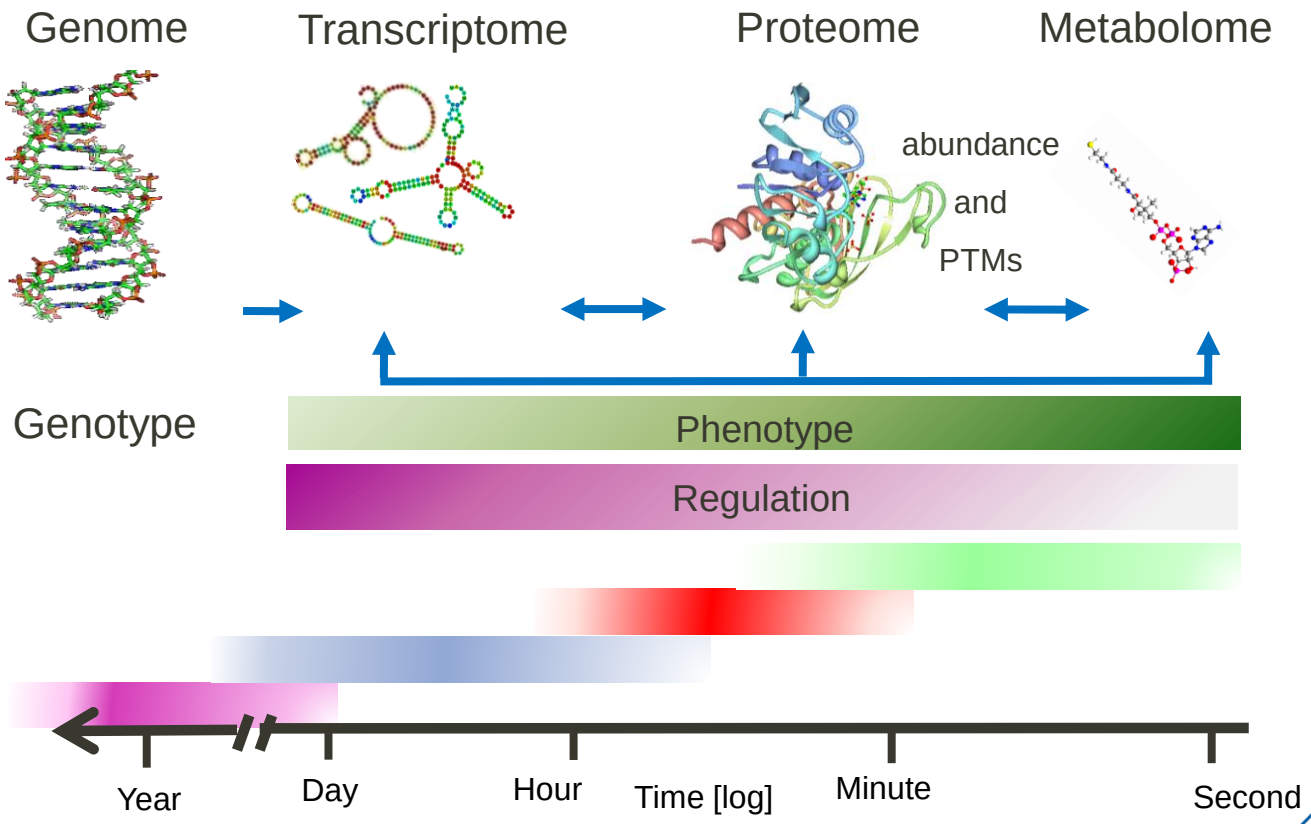
https://www.bfr-akademie.de/english/events/nam-omics.html

Two reasons to use omics for hazard assessment

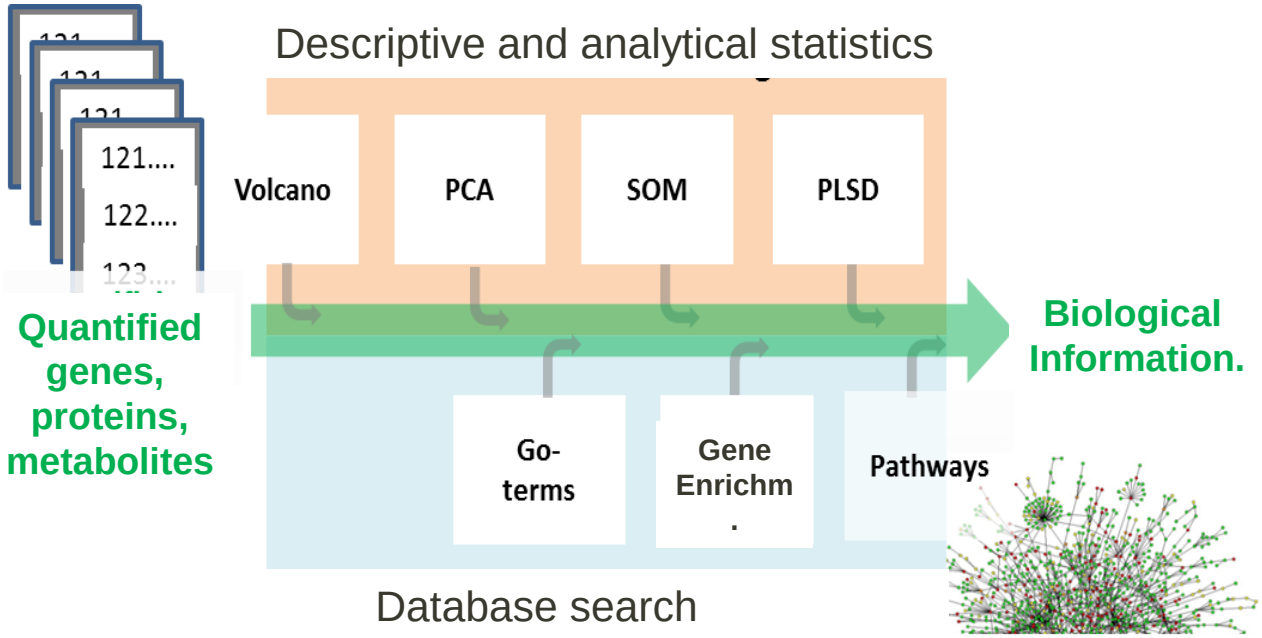


1. Sensitive detection of hazardous effects
2. Identification of molecular initiating and key events for AOPs

Information and time scale hierachy in biochemistry

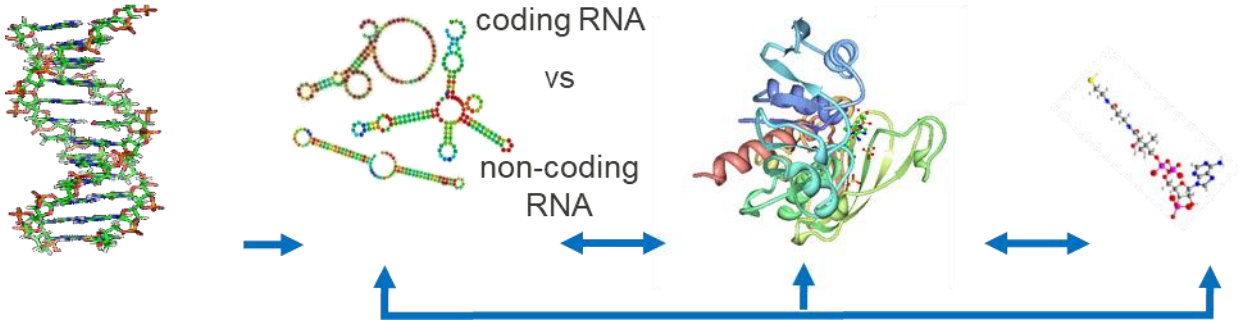


From data to biological information



- Every step has been optimized for an omics technique

Chain of evidence is completed by combination of omics



Information on mRNA
„Which protein functions could be there“

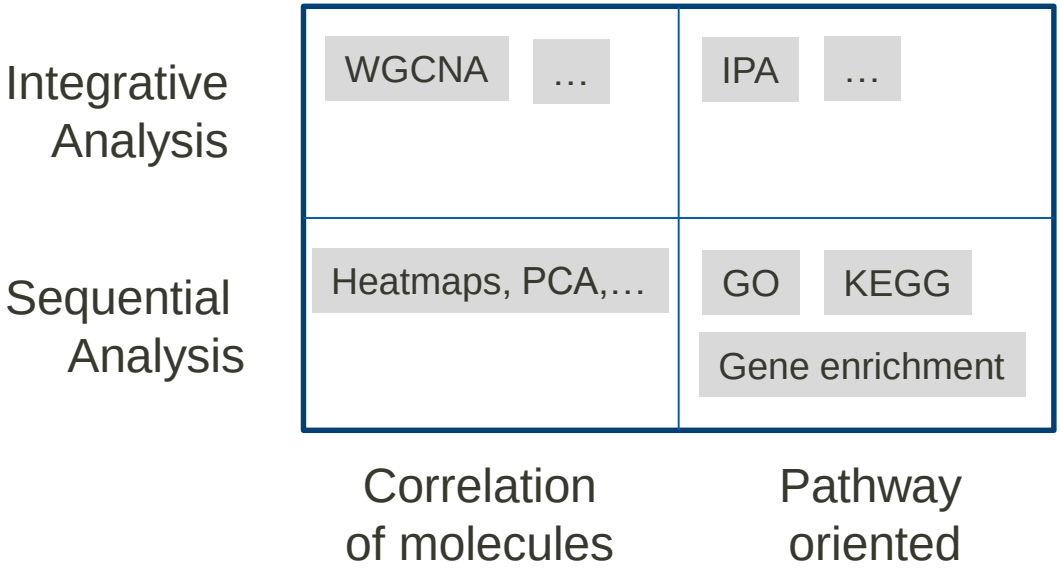
Information on proteins
„Which proteins are there“
But abundance ≠ function

Information on metabolites
„Which functions are carried out“

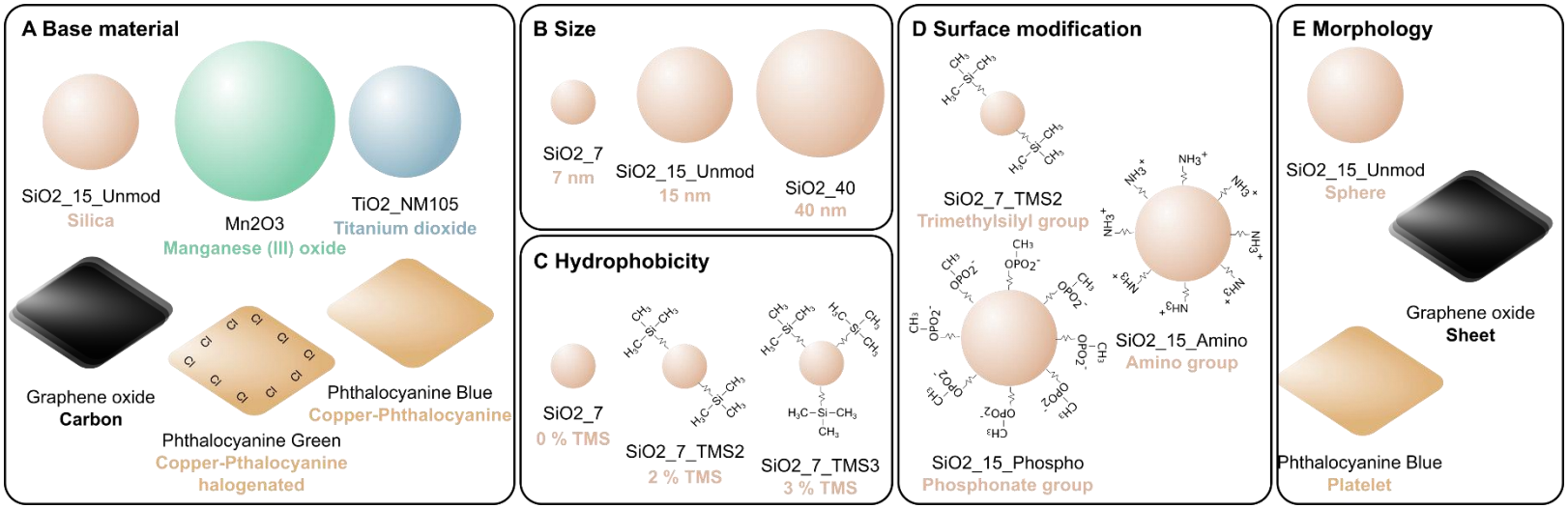
- In combination with information on the cellular or organ phenotype the underlying pathways can be inferred
- With the existing methods it is now possible to obtain a (more) complete chain of evidence

→ Combination ≠ integration

Integration of omics data requires other approaches

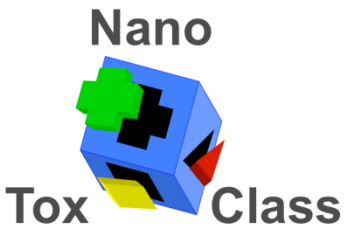


Integration of omics in hazard assessment of NMs

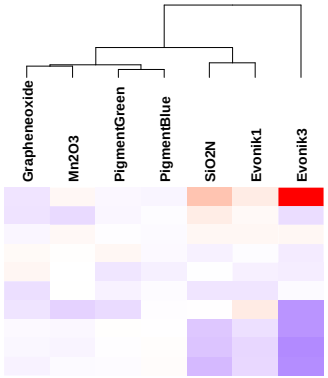


Variation in

- → Primary particle sizes
- → Agglomerate sizes
- → Base materials
- → Morphology



Omics provides more parameters and mechanistic insights



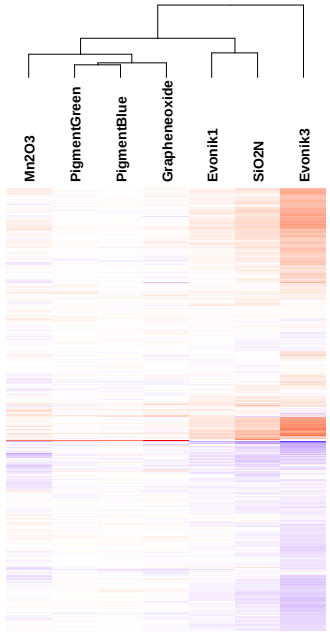
Single Parameter based Grouping

LDH

WST

ROS

...



OMICS based Grouping

> 1000 parameters

More measured parameters lead to higher resolution

→ **OMICS data allow more reliable grouping**

OMICS data provide information about mode of action

→ **Mechanism based grouping possible**

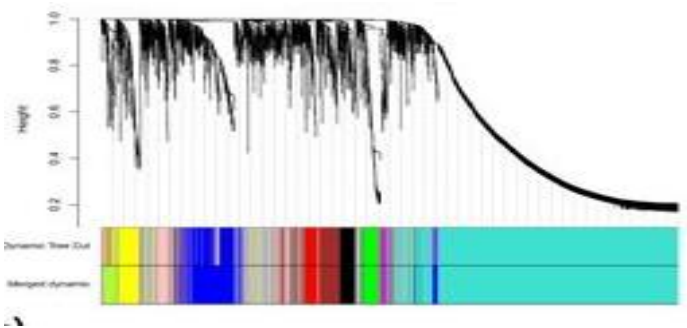
The people who did the work

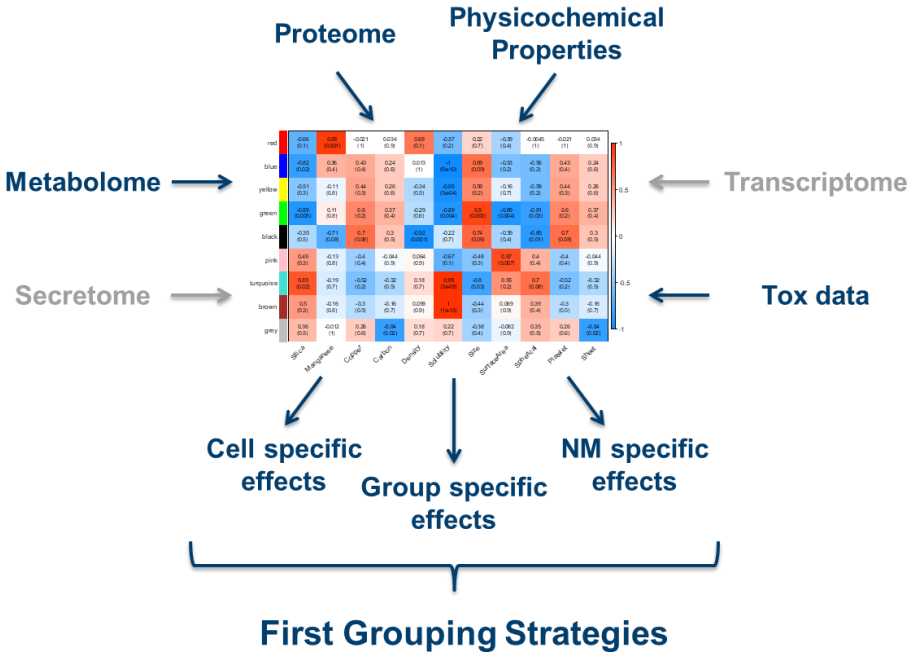
A nanotox example

	GO/KEGG Enrichment	IPA	WGCNA
P-value independent analysis	X	X	✓
FC dependent analysis	X	✓	✓
Incorporation of small changes	X	X	✓
Correlation to treatments	X	X	✓
Enrichment Analysis	✓	✓	X

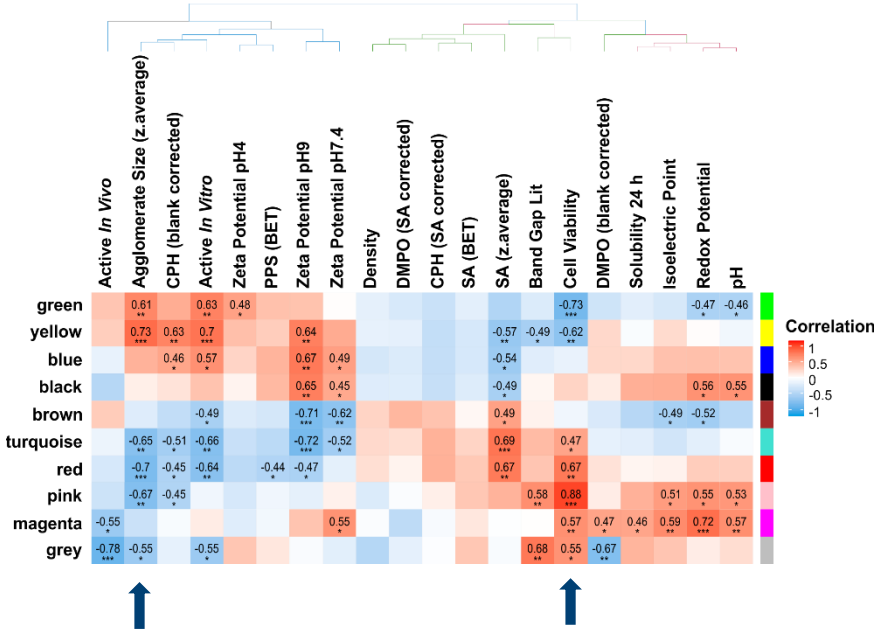
GENEONTOLOGY Unifying Biology

QIAGEN





Correlations with physical and toxicological parameters

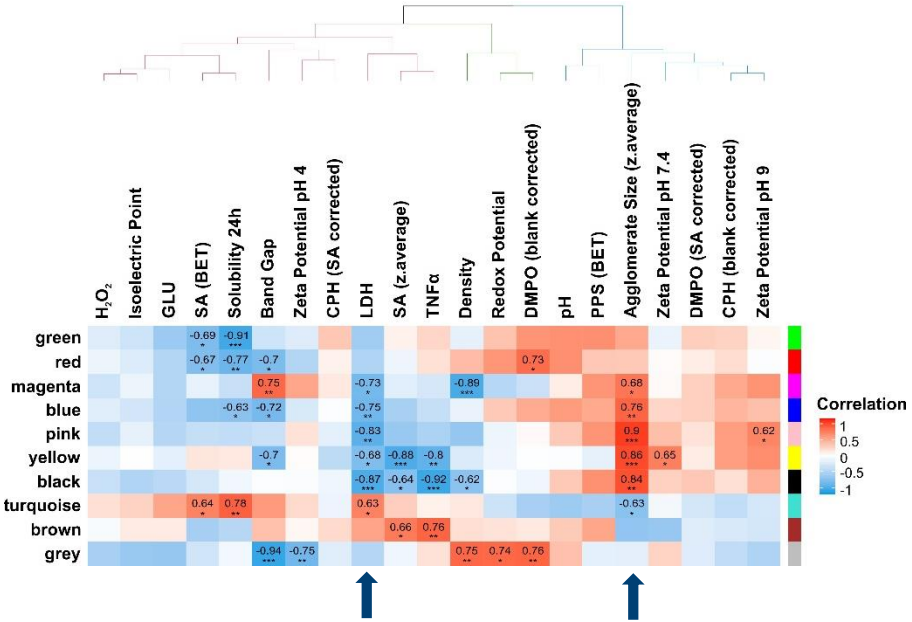


Alveolar epithelial cells

Agglomerate size and cell viability show strong correlation with molecular changes

Karkossa et al., Part Fibre Toxicol. 2019 Oct 25;16(1):38

Correlations with physical and toxicological parameters

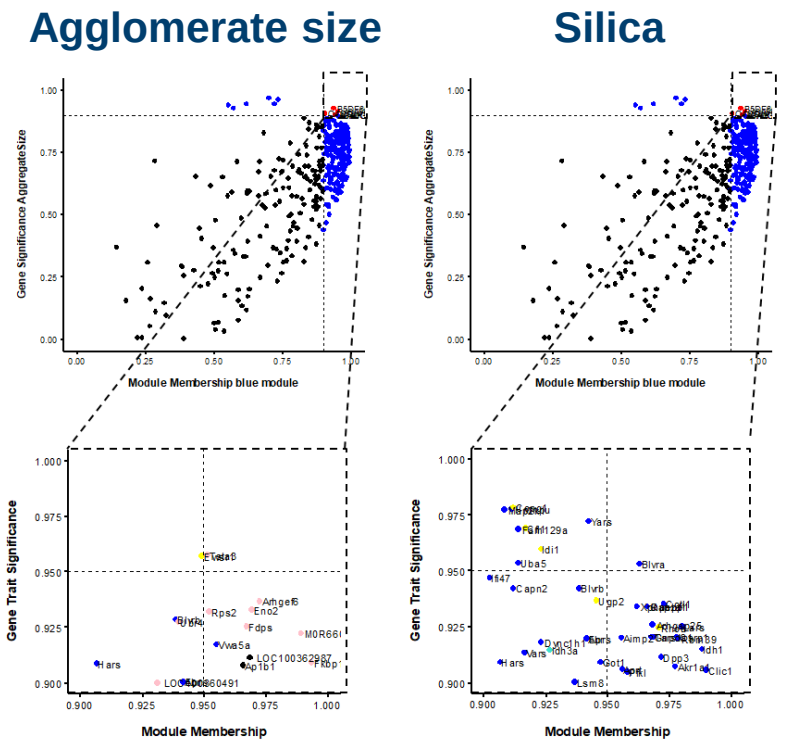


Alveolar macrophages

Agglomerate size and LDH release show strongest correlation with molecular changes

Bannuscher et al., Nanotoxicology 2020;14(2):181-195

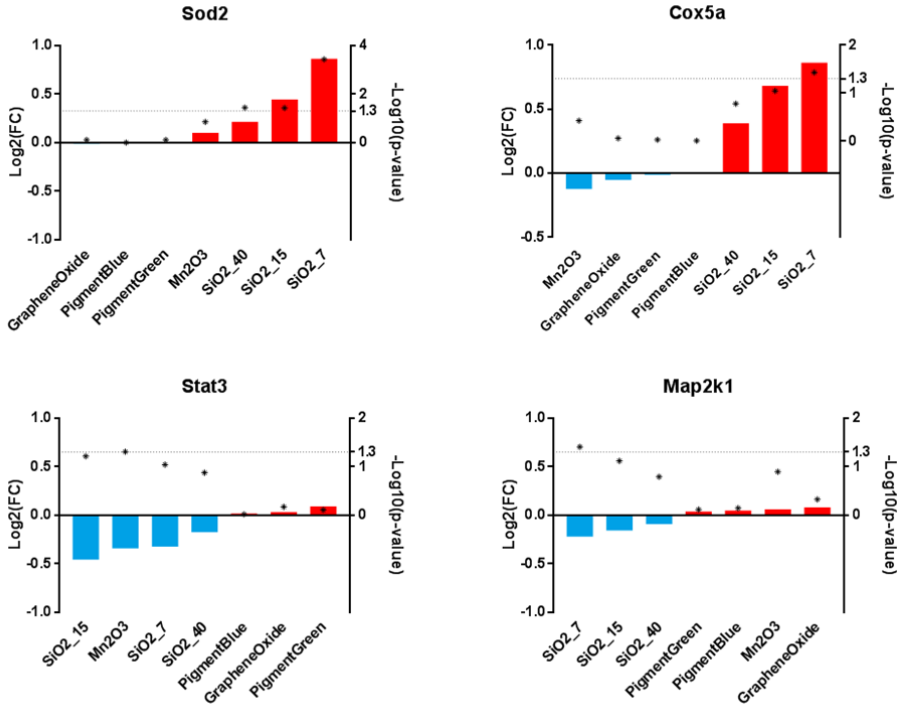
Identification of key drivers



- → **Plotting GS (gene significance) against MM (module membership)**
  - → GS: correlation of expression profiles with physicochemical properties
  - → MM: correlation of expression profiles with module eigengenes
- → **Identification of highly connected analytes**
  - → Gene significance ≥ 0.9
  - → Module membership ≥ 0.9
- → **Selection of key drivers from all highly correlating modules**

Bannuscher et al., Nanotoxicology 2020;14(2):181-195

Key drivers allow grouping of nanomaterials

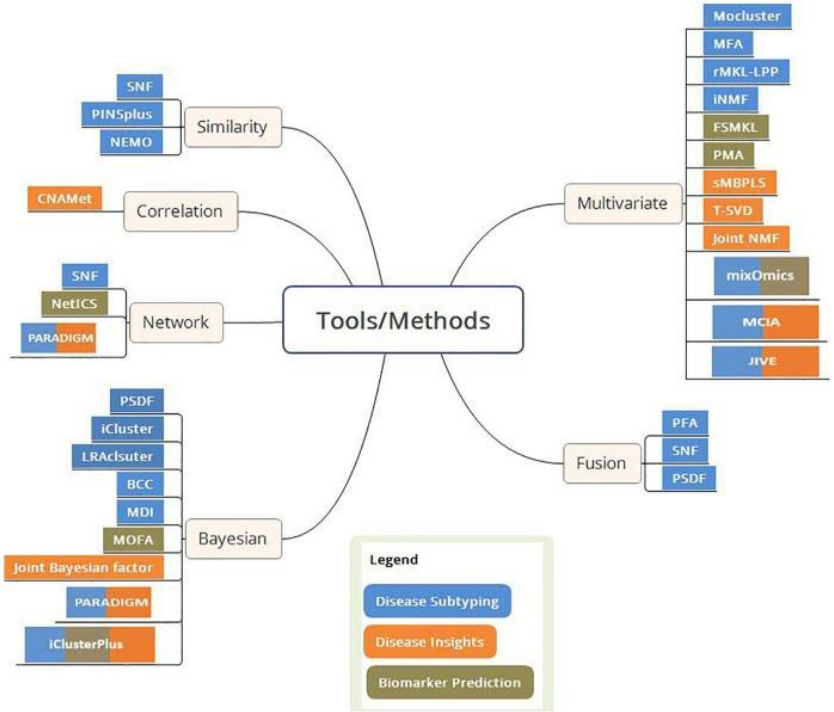


Two groups observable

Silica NMs **Other NMs**

Bannuscher et al., Nanotoxicology 2020;14(2):181-195

There are many more existing software tools



- → **A wide range of methods are actually developed with different strengths and weaknesses**
- → **The optimized choice depends strongly on the research question**

Subramanian et al., Multi-omics Data Integration, Interpretation, and Its Application Bioinform Biol Insights. 2020; 14: 1177932219899051.

Conclusions (I)

OMICS techniques allow the determination of the mode of action of NMs

→ Integrative analysis of several OMICS data sets results in a more comprehensive overview

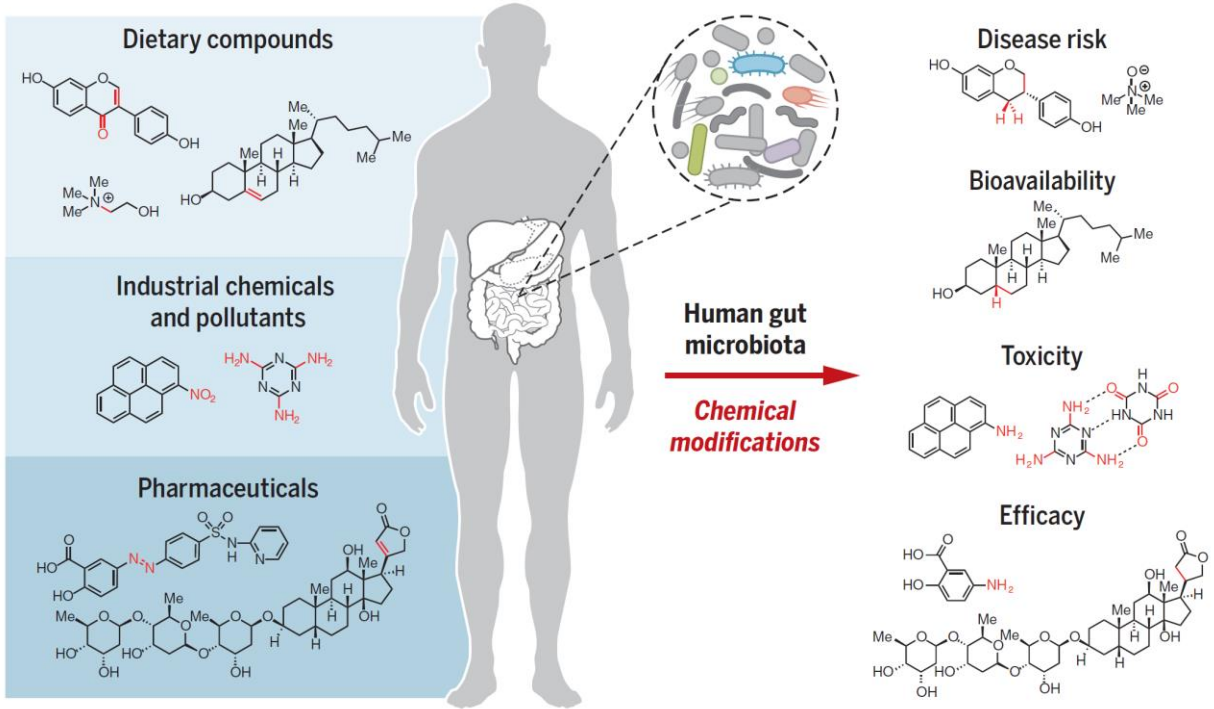
Weighted Gene Correlation Network Analysis allows the correlation to treatments as well as physicochemical properties and tox data

→ Facilitates development of grouping strategies

Key drivers are identified based on Weighted Gene Correlation Network Analysis results

→ Suitable biomarker candidates for future risk assessment

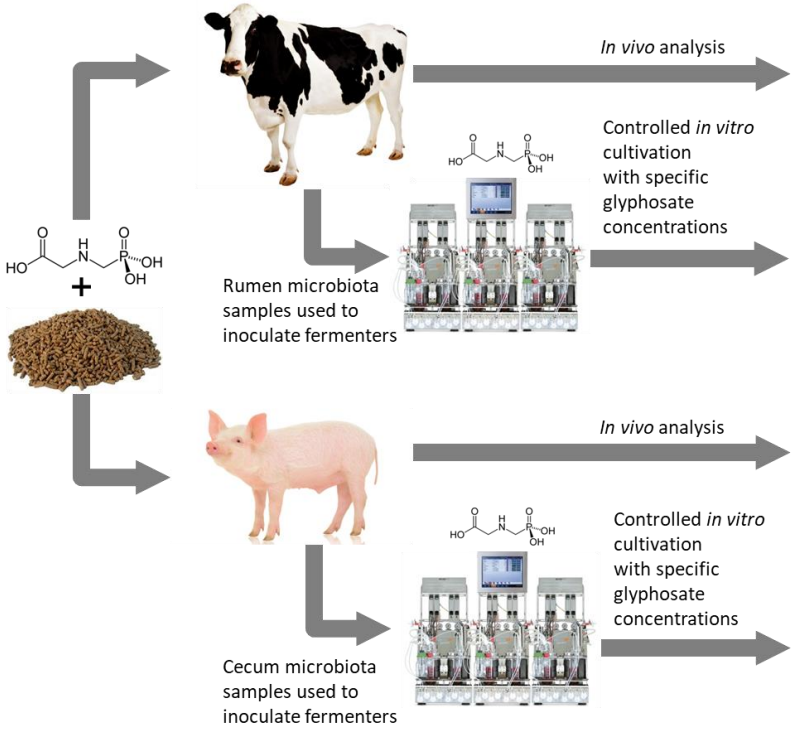
Microbiome dependent toxicity



Koppel N *et al.*, Science (2017)

Effects of Glyphosate on the microbiome of pigs and cows

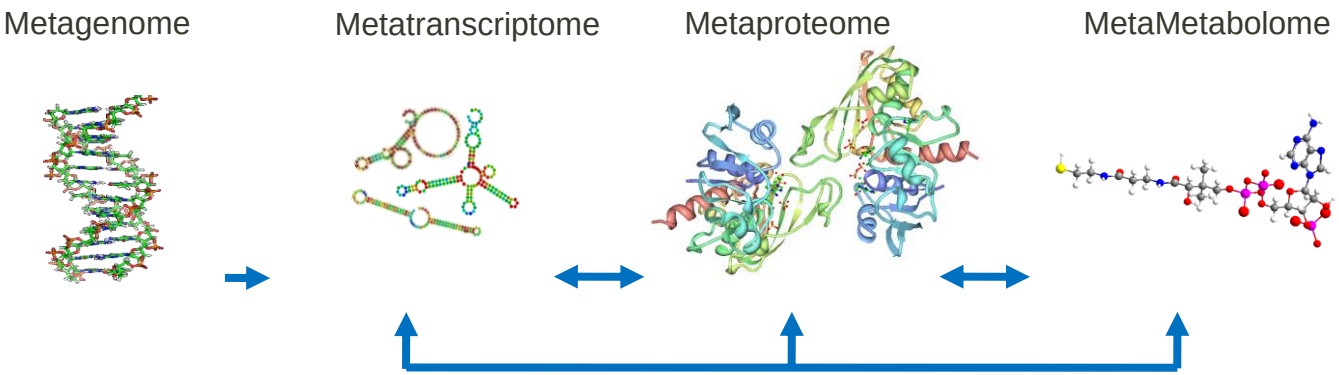
- In 2017 glyphosate was heavily discussed in EU
- Due to the presence of the EPSPS in bacteria an impact on the microbiome was discussed
- BMEL funded GlyphoBac-project on the effects of glyphosate on the intestinal microbiome of cows and pigs



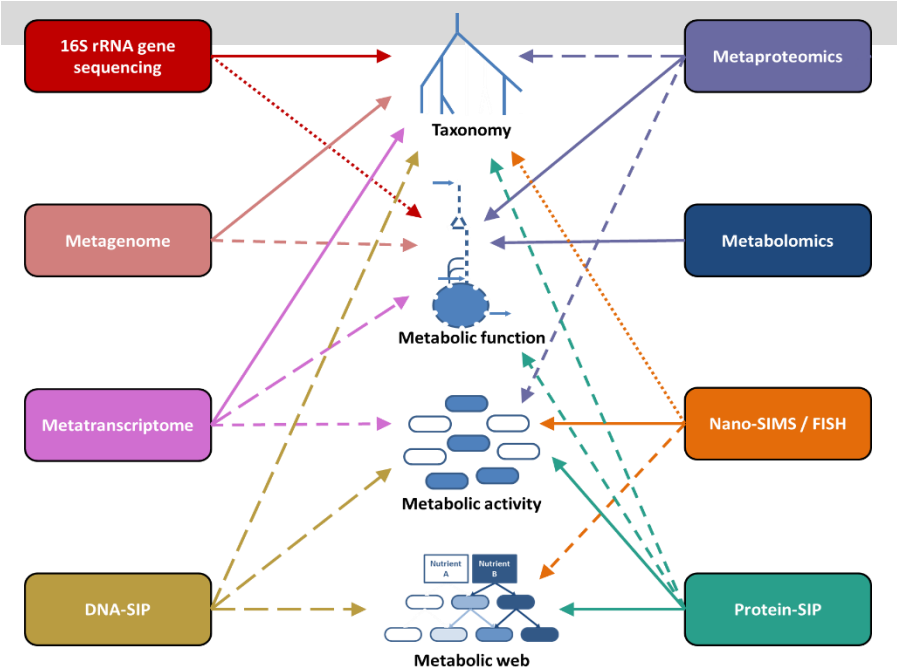
Our tasks: Molecular analytics for project partners and own fermenter samples

- Determining glyphosate and its metabolites in feces, blood and urine
- Cultivation of pig microbiota samples under controlled conditions in fermenters with glyphosate
- 16S rRNA gene seq. analysis of samples
- Metaproteomics of samples
- Metagenomics of samples
- $^{13}C/^{15}N$ labelled flux analysis of own fermenter samples

Geno-, phenotype, and taxonomic information by metaomics



Meta-omics: information gained by methods



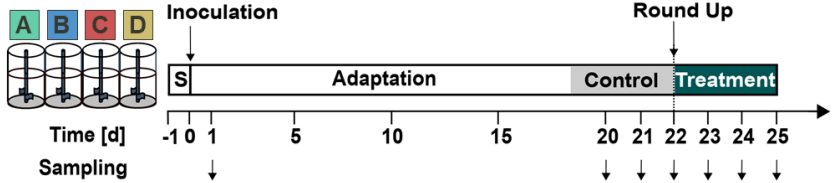
- Full lines depict the maximum amount of information which can be obtained, with the dashed lines signifying less information obtainable and the smallest dashes signifying least information obtainable from the method
- Generally a multi-omics approach is ideal because the methods complement each other well

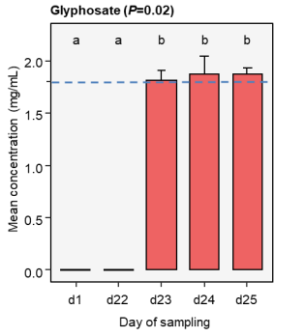
The people who did the work

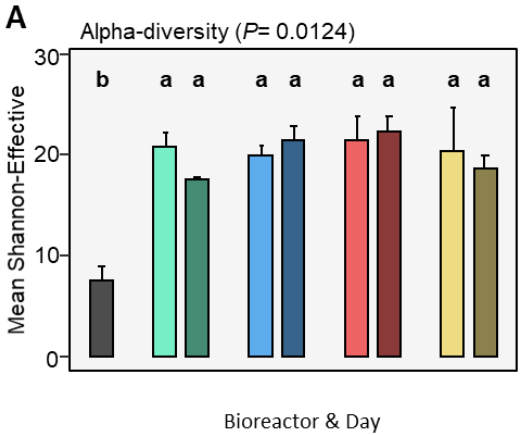
- Jannike Krause
- Nico Jehmlich
- Katharina Fritz-Wallace
- Jean Fromment

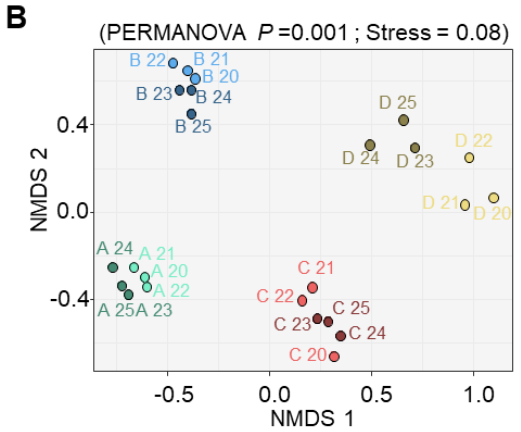
Effects of Glyphosate on pig microbiome

- Method for Glyphosate detection was established
- Bioreactors are an effective option to analyse the effects of chemicals on the microbiome
- 16SrRNA analysis provides information on the community



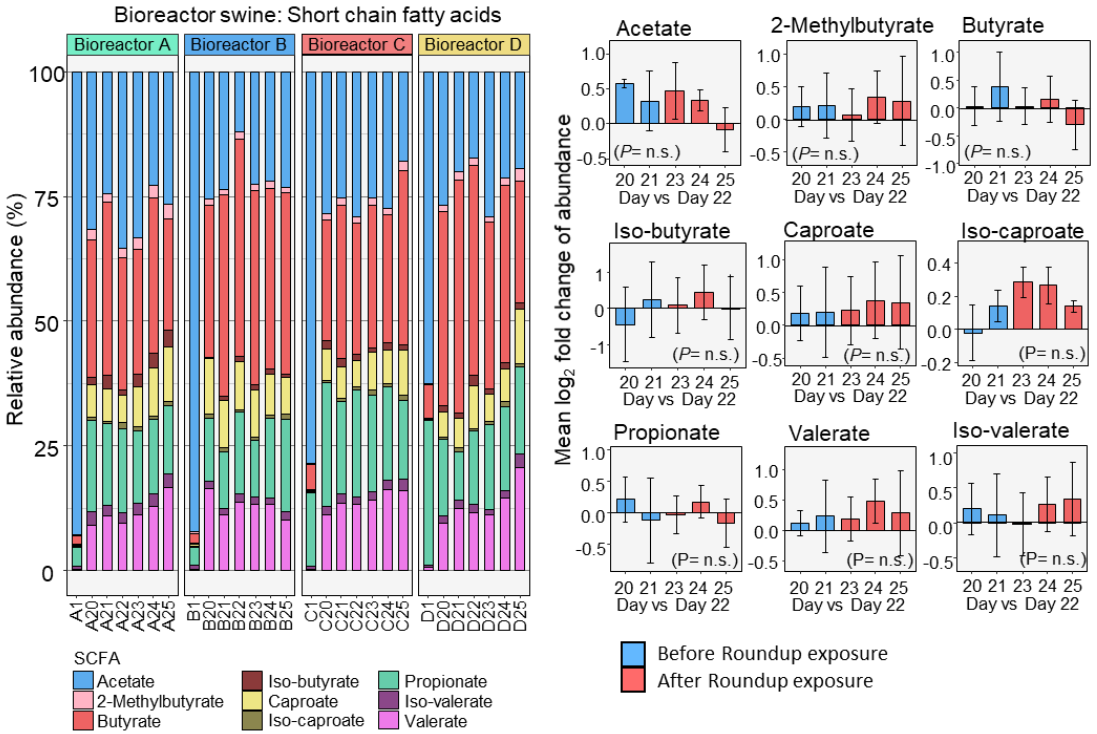






Fritz-Wallace K et al., Rapid Commun Mass Spectrom. 2020 Apr 15;34(7)

Glyphosate does not affect the SCFA metabolism



- SCFAs are key for characterising the overall metabolic activity of the microbiome
- There is no effect of glyphosate detectable
- There is neither an effect on the metaproteome level

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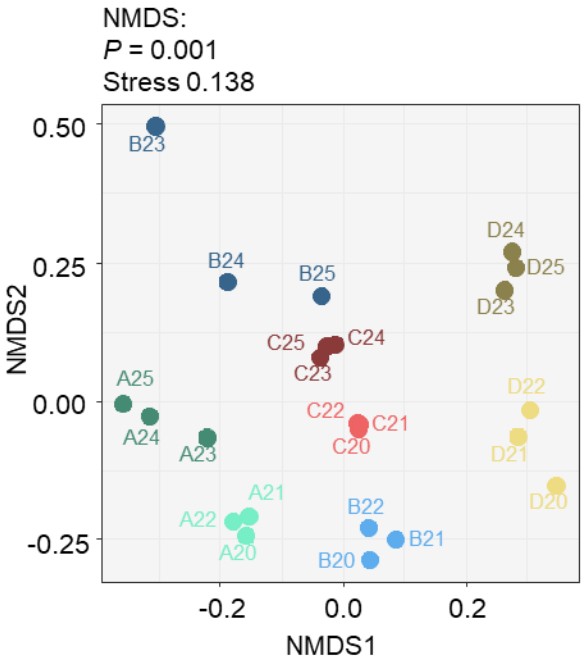
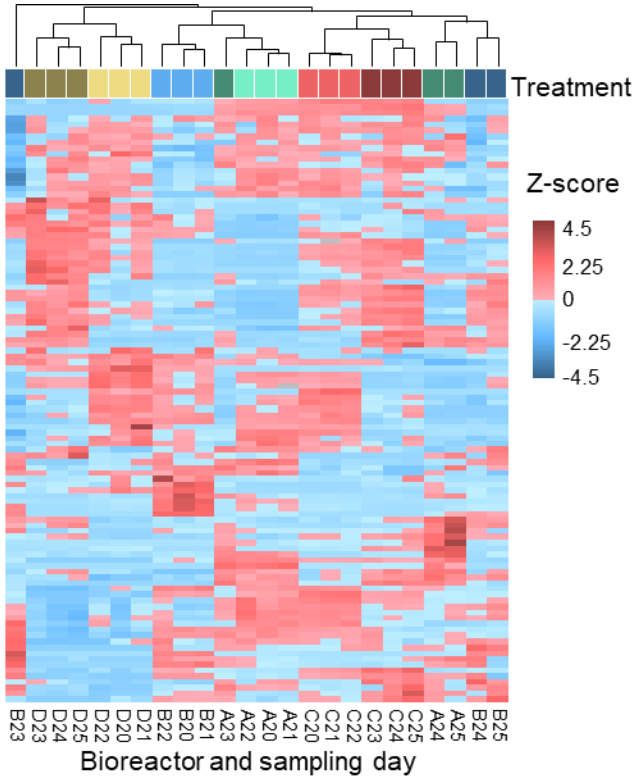
Krause et al., Sci Total Environ. 2020 Nov 25;745:140932

Glyphosate affects the metabolism very subtly

- Untargeted metabolomics detects subtle effects





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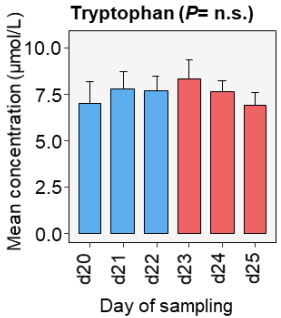
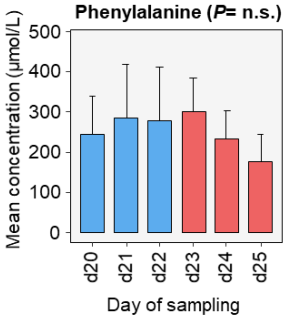
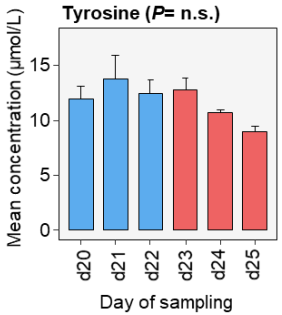
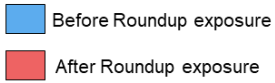
Krause et al., Sci Total Environ. 2020 Nov 25;745:140932

Aromatic amino acids might prevent EPSPS dependent effects









- Tryptophan and tyrosine were present in concentrations around 10 µmol and might have suppressed the expression of EPSPS
- Our results show that there only very subtle effects of glyphosate in this model
- It is possible that under protein-limited conditions like the transition from weaning to solid food APSSS dependent effects can occur

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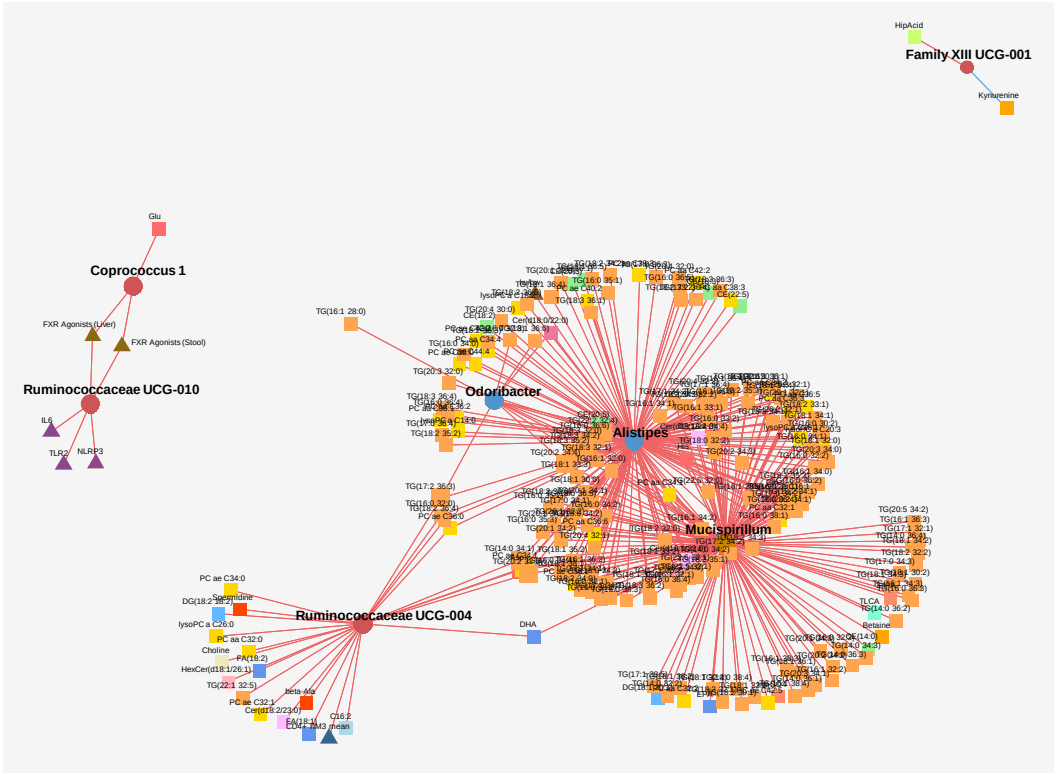
Krause et al., Sci Total Environ. 2020 Nov 25;745:140932

Correlation networks can also be used for integration of metaomics data



Group

- Actinobacteria
- Acylcarnitines
- Aminoacids
- Aminoacids Related
- Bacteroidetes
- Bile Acids
- Bile_acids
- Carboxylic Acids
- Ceramides
- Cholesterol Esters
- Diacylglycerols
- Dihydroceramides
- FACS_limphoid
- FACS_myeloid
- Fatty Acids
- Firmicutes
- Glycerophospholipids
- Glycosylceramides
- Physiological_parameters
- Proteobacteria
- RT_qPCT
- Serum_values
- Sphingolipids
- Triacylglycerols
- Verrucomicrobia
- Vitamins & Cofactors

Group

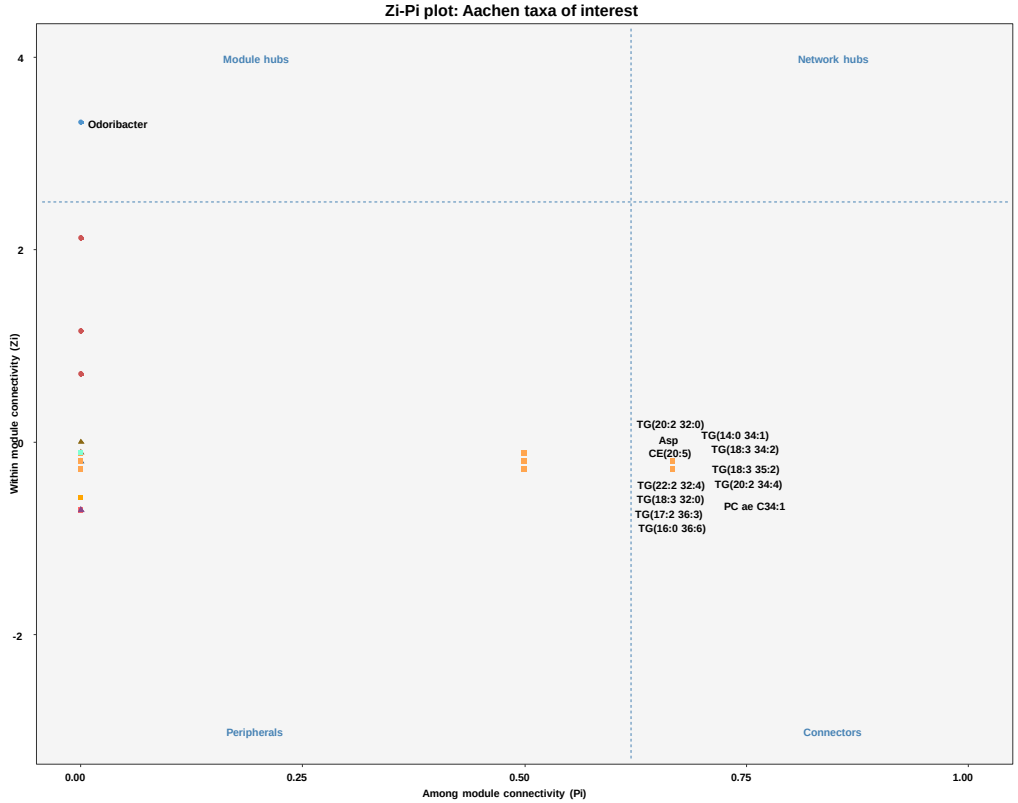
- Bacteria
- Clinical_parameters
- Metabolite

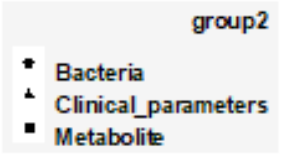
Degree

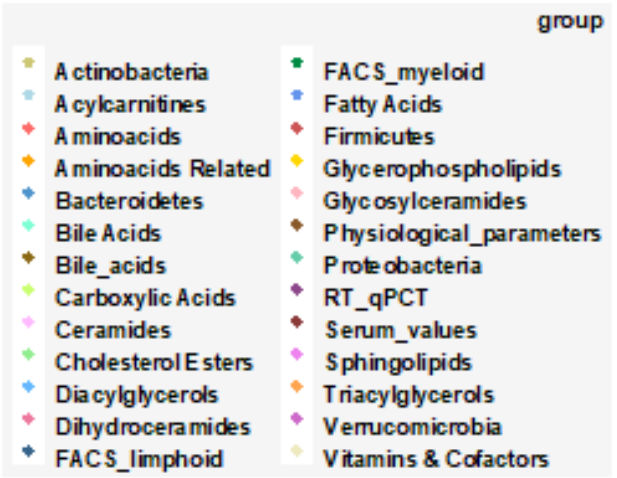
- [2,4]
- (4,6]
- (6,8]
- (8,262]

Red edges positive associations, blue edges negative associations

Z / Pi plot of Network analysis Correlation network of genera with significantly altered abundances with metabolite concentrations and clinical parameters







Conclusions

- Our results show that there only very subtle effects of glyphosate in this model of pig microbiome

- Correlation analysis is also a suitable tool for integration of metaomics data and with other phenotypical information

Acknowledgements

Nanotox Class
Andrea Haase (BfR)
Andreas Luch (BfR)

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Gunda Herberth
Jannike Krause

NRLP6-Project
Christian Trautwein (UK Aachen

CRC1382 **GUT-LIVER AXIS**
FUNCTIONAL CIRCUITS AND THERAPEUTIC TARGETS

Department Molecular Systems Biology

Functional Genomics
Kristin Schubert
Isabel Karkossa

Functional Metabolomics
Ulrike Rolle-Kampczyk
Beatrice Engelmann
Sven Haange

Thanks for your attention
…..and the following agencies for funding

DFG Deutsche Forschungsgemeinschaft

Europa fördert Sachsen. EFRE Europäischer Fonds für regionale Entwicklung

SFB 1052 Obesity Mechanisms

INTESTINAL MICROBIOTA

Nano Tox Class

novo nordisk®

MATOMIC

CRC1382 GUT-LIVER AXIS
FUNCTIONAL CIRCUITS AND THERAPEUTIC TARGETS

Bundesministerium für Ernährung und Landwirtschaft

SPP 2002

DBU Deutsche Bundesstiftung Umwelt

cefic

nano/nformaTIX

SPP 1319

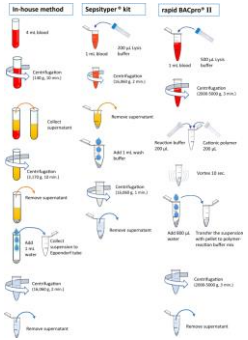
AquaDiva

DAAD Deutscher Akademischer Austauschdienst German Academic Exchange Service

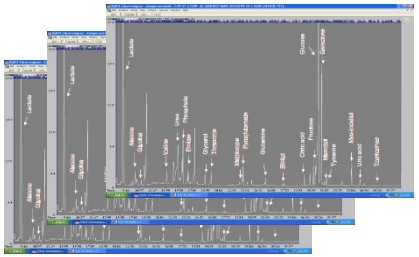
BfR Bundesinstitut für Risikobewertung

What are the most relevant hurdles for implementing omics in risk assessment?

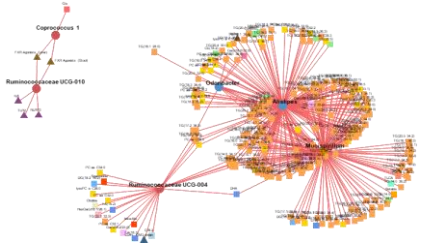
Sampling



Sample prep.



Measurement



Bioinformatics

1. Repeatability and Reproducibility
2. Research Data Management

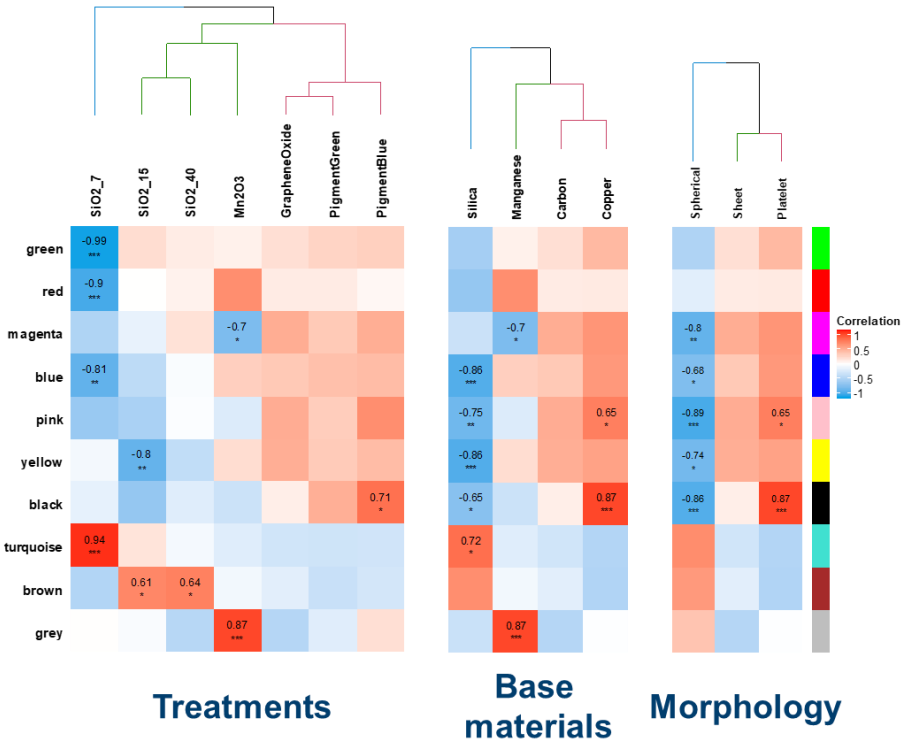
Acknowledgements

NanotoxClass

BMEL

GlyphoBaC

Papiere hinzufügen



Module	#Proteins	#Metabolites
green	44	1
red	41	0
magenta	29	0
blue	336	20
pink	28	1
yellow	60	0
black	38	0
turquoise	555	7
brown	54	63
grey	15	0

→ **Determination of silica specific effects possible**